TOWARDS THE DEVELOPMENT OF A YEAST-BASED OPIOID BIOSENSOR

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Biosensors can be applied as powerful screening tools to quickly and efficiently optimize synthetic metabolic pathways, an essential process for the development of strains with commercially viable yields. In particular, an opioid biosensor is needed to accelerate the development of opioid-producing yeast strains and the search for non-euphoric, analgesic compounds that could replace opioids as pain management tools. We are constructing such a biosensor by linking opioid receptors to the pheromone response pathway of the yeast *S. cerevisiae*. Opioid receptors are plasma membrane localized G protein-coupled receptors (GPCRs), some of which have successfully been linked to the pheromone response pathway through chimeric alpha G protein subunits. Adding complexity, functional opioid receptors require the presence of the animal sterol cholesterol as opposed to the fungal sterol ergosterol. We have successfully engineered a cholesterol-producing yeast with alpha subunit chimeras and optimized GFP-based reporters but have not yet detected activity upon introduction of opioid receptors.

Now, using microscopy-based approaches, we are highlighting proper localization of exogenous receptors as an under-reported road block in yeast biosensor development. By tagging a subset of opioid receptors with green fluorescent protein we demonstrate that while expressed, opioid receptors are restricted to the endoplasmic reticulum (ER) independent of which sterol is present. They are either dispersed throughout the ER or in puncta that colocalize with the early Golgi marker Cop1. In an effort to induce ER-export, we have systematically generated mutations in the human mu-opioid receptor and tested mutations in yeast quality control machinery. Our ongoing efforts highlight novel strategies to properly localize exogenous membrane proteins in yeast for biosensor development.